Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/006822

International filing date: 01 March 2005 (01.03.2005)

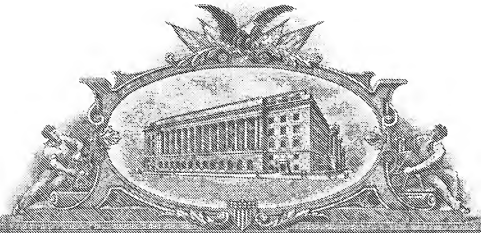
Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/549409
Filing date: 01 March 2004 (01.03.2004)

Date of receipt at the International Bureau: 25 April 2005 (25.04.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse



THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

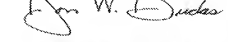
United States Patent and Trademark Office

April 18, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: ***60/549,409***
FILING DATE: ***March 01, 2004***
RELATED PCT APPLICATION NUMBER: ***PCT/US05/06822***

Certified by



Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

20427 U.S. PTO
030104

Express Mail Label: EV392153493US
Date of Deposit: March 1, 2004

Attorney Docket No. 24655-021

22264 U.S. PTO
60/549409
030104

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR § 1.53(c)

INVENTORS / APPLICANTS

Given Name (last name, first name, and middle initial [if any]			Residence (City and State or Foreign Country)
Strominger	Jack	L.	Lexington, Massachusetts

TITLE OF THE INVENTION

COPOLYMERS FOR SUPPRESSION OF DIABETES AND METHODS OF USE

CORRESPONDENCE ADDRESS

Attorney Name: Firm Name and Address:	Sonia K. Guterman, Ph.D., J.D. MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C. One Financial Center Boston, MA 02111
Telephone:	(617) 542-6000
Fax:	(617) 542-2241

ENCLOSED APPLICATION PARTS

- ☒ **Specification** — Number of Pages: 35
- ☐ **Sequence Listing** — Number of Pages:
- ☐ **Drawings** (☐ Formal; ☐ Informal) — Number of Sheets:
- ☐ **Other documents** (specify):

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government:

- ☐ No.
- ☒ Yes, the name of the U.S. Government agency and the Government contract number are: National Institutes of Health – Grants DK57730 and AI49524

METHOD OF PAYMENT

- ☒ A check in the amount of **$80.00** is enclosed to cover the filing fees of the Provisional application.
- ☐ The Commissioner is hereby authorized to charge **$80.00** is enclosed to cover the filing fees of the Provisional application to Deposit Account No. 50-0311, Reference No. 24655-021.
- ☒ The Commissioner is hereby authorized to charge additional fees or credit any overpayment to Deposit Account No. 50-0311, Reference No. 24655-021.

Respectfully submitted,

Sonia K. Guterman

Date: March 1, 2004

Sonia K. Guterman, Esq., Reg. No. 44,729
MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C.
One Financial Center
Boston, MA 02111
Telephone: (617) 542-6000
Customer No. 30623

Attorney Docket: 24655-021

COPOLYMERS FOR SUPPRESSION OF DIABETES, AND METHODS OF USE

Technical Field

The invention relates to design of copolymers having particular amino acids in specific molar ratios, synthesized into polypeptides of predetermined length and capable of suppression of symptoms and frequency of recurrent episodes of an autoimmune disease, particularly insulin dependent diabetes mellitus (IDDM), also called type I diabetes (TID).

Government Funding

This invention was made in part with funding under grants DK57730 and AI49524 from the National Institutes of Health. The government has certain rights in the invention.

Background

Type 1 or insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease resulting in selective destruction of the islet ß cells in the pancreas. In both the human and nonobese diabetic (NOD) mice, glutamic acid decarboxylase (GAD 65) has been suggested as a dominant autoantigen in the pathogenesis of the disease. The susceptibility to IDDM is associated with class II major histocompatibility complex (MHC) genes. In humans, HLA-DR4, HLA-DQ8 and HLA-DR3, HLA-D2 haplotypes predispose subjects to the disease. Most data suggest that DQ rather than DR alleles are strongly associated with susceptibility to IDDM, but in some populations the linkage to DR has been observed. In a mouse model system of diabetes, naturally processed peptides derived from GAD65 are bound to HLA-DQ8 and/or HLA-DR3 protein in spleens and lymph nodes of Aß^{o}/DQ3, DQ8 transgenic animals, which develop insulitis and glutamic acid decarboxylase (GAD65) autoreactivity spontaneously.

Copolymer 1 (Copl; Copaxone®; YEAK) is a random amino acid copolymer of alanine (A), lysine (K), glutamic acid (E) and tyrosine (Y) in a molar ratio of approximately 5:3:1.5:1. Copl is synthesized in solution using N-carboxyamino acid anhydrides (Teitelbaum D. et al. 1971. *Eur. J. Immunol.* 1:242-248). Initially, this and other related copolymers were used to define the genetic basis of immune responsiveness, now known as class II MHC genes (McDevitt, H.O., and M. Sela. 1965. *J. Exp. Med.* 122:517-532; McDevitt, H.O., and M. Sela. 1967. *J. Exp. Med.* 126:969-978). Copl, also known as poly $(Y,E,A,K)_n$ or YEAK was found to

be effective both in suppression of experimental allergic encephalomyelitis (Teitelbaum D. et al. 1971. *Eur. J. Immunol.* 1:242-248; Teitelbaum D. et al. 1973. *Eur. J. Immunol.* 3:273-279; Teitelbaum D, et al. 1974; *Clin. Immunol. Immunopathol.* 3:256-262; Aharoni R. et al. 1993. *Eur. J. Immunol.* 23:17-25) and in the treatment of relapsing forms of multiple sclerosis (MS; Bornstein, M.B. et al. 1987. *N. Engl. J. Med.* 317:408-414; Johnson, K.P. et al. 1995. *Neurology* 45:1268-1276; Johnson, K.P. et al. 1998. *Neurology* 50:701-708).

Copl has been approved as a therapy for MS and currently is in wide use. However, while Copl reduces the MS relapse rate, it does not treat diabetes. It is important to develop improved compositions and methods of use for treatment of diabetes, and for other autoimmune diseases that may be linked to DQ2 or DQ8.

Summary

An embodiment of the invention is a copolymer composition comprising a random sequence of at least three different amino acid residues wherein at least one amino acid is selected from each of: a first group of amino acids consisting of glutamic acid (E), aspartic acid (D), glutamine (Q) and asparagine (N); a second group of amino acids consisting of valine (V), leucine (L), threonine (T), serine (S), and isoleucine (I); and a third group of amino acids consisting of alanine (A) and glycine (G).

Accordingly, the copolymer is for example a terpolymer comprising amino acid residues which is selected from the group consisting of: aspartic acid:alanine:leucine (DAL); aspartic acid:alanine:valine (DAV); aspartic acid:alanine:threonine (DAT); aspartic acid:alanine:serine (DAS); asparagine:alanine:leucine (NAL); asparagine:alanine:valine (NAV); asparagine:alanine:threonine (NAT); asparagine:alanine:serine (NAS); glutamic acid:alanine:leucine (EAL); glutamic acid:alanine:valine (EAV); glutamic acid:alanine: threonine (EAT); glutamic acid:alanine: serine (EAS); glutamine:alanine:leucine (QAL); glutamine:alanine:valine (QAV); glutamine:alanine: threonine (QAT); glutamine:alanine: serine (QAS); aspartic acid:alanine:isoleucine (DAI); glutamic acid:alanine:isoleucine (EAI); aspartic acid:glyine:isoleucine (DGI); aspartic acid:glyine: threonine (DGT); aspartic acid:glyine: serine (DGS); asparagine:alanine:isoleucine (NAI); glutamine:alanine:isoleucine (QAI); asparagine:glyine:isoleucine (NGI); asparagine:glyine: threonine (NGT); asparagine:glyine: serine (NGS); glutamic acid:glyine:isoleucine (EGI); aspartic acid:glyine:valine (DGV); glutamic acid:glyine:valine (EGV); glutamic acid: glyine:leucine (EGL); glutamic acid:

glyine:threonine (EGT); glutamic acid: glyine:serine (EGS); glutamic acid: glyine:valine (EGV); glutamine:glyine:isoleucine (QGI); asparagine:glyine:valine (NGV); glutamine:glyine:valine (QGV); glutamine: glyine:leucine (QGL); glutamine: glyine:threonine (QGT); glutamine:glyine:serine (QGS); and glutamine:glyine:valine (QGV).

In general in the terpolymer compositions, the copolymers are synthesized to have a molar ratio of the amino acid components is about 2:5:3 for relative amounts of amino acids of the first group, the second group, and the third group, respectively. Alternatively, the molar ratio of the amino acid components is about 2:25:15 for relative amounts of amino acids of the first group, the second group, and the third group, respectively. Alternatively, the molar ratio of the amino acid components is about 2:1:0.6 for relative amounts of of the first group, the second group, and the third group, respectively.

In an alternative embodiment, the copolymer is a tetrapolymer selected from the group comprising amino acid residues aspartic acid:alanine:leucine:glutamic acid (DALE); asparagine:alanine:leucine:glutamic acid (NALE); aspartic acid:alanine:leucine:glutamine (DALQ); aspartic acid:alanine:valine: glutamic acid (DAVE); asparagine:alanine:valine: glutamic acid (NAVE); aspartic acid:alanine:valine: glutamine (DAVQ); aspartic acid:alanine:isoleucine:glutamic acid (DAIE); asparagine:alanine:isoleucine:glutamic acid (NAIE); aspartic acid:alanine:isoleucine:glutamine (DAIQ); aspartic acid:alanine:threonine:glutamic acid (DATE); asparagine:alanine:threonine:glutamic acid (NATE); aspartic acid:alanine:threonine:glutamine (DAQE); aspartic acid:alanine:serine:glutamic acid (DASE); asparagine:alanine:serine:glutamic acid (NASE); aspartic acid:alanine:serine:glutamine (DASQ); aspartic acid:glyine:isoleucine:glutamic acid (DGIE); asparagine:glyine:isoleucine:glutamic acid (NGIE); aspartic acid:glyine:isoleucine:glutamine (DGIQ); glutamic acid:glyine:leucine:glutamic acid (DGLE); glutamine:glyine:leucine:glutamic acid (QGLE); aspartic acid:glyine:leucine:glutamine (DGLQ); aspartic acid:glyine:threonine: glutamic acid (DGTE); asparagine:glyine:threonine:glutamic acid (NGTE); aspartic acid:glyine:threonine: glutamine (DGTQ); aspartic acid:glyine:serine: glutamic acid (DGSE); asparagine:glyine:serine: glutamic acid (NGSE); aspartic acid:glyine:serine: glutamine (DGSQ); aspartic acid:glyine:valine:glutamic acid (DGVE). asparagine:glyine:valine:glutamic acid (NGVE); and aspartic acid:glyine:valine:glutamine (DGVQ).

In alternative embodiments the invention provides: a copolymer composition comprising a random sequence of amino acid residues aspartic acid, alanine, leucine, and glutamic acid (DALE); a copolymer composition comprising a random sequence of amino acid residues aspartic acid, alanine, valine, and glutamic acid (DAVE); a copolymer composition comprising a random sequence of amino acid residues aspartic acid, alanine, threonine, and glutamic acid (DATE); a copolymer composition comprising a random sequence of amino acid residues aspartic acid, alanine, isoleuine, and glutamic acid (DAIE); a copolymer composition comprising a random sequence of amino acid residues aspartic acid, glycine, leucine, and glutamic acid (DGLE); a copolymer composition comprising a random sequence of amino acid residues aspartic acid, glycine, valine, and glutamic acid (DGVE); a copolymer composition comprising a random sequence of amino acid residues aspartic acid, glycine, isoleucine, and glutamic acid (DGIE); and a copolymer composition comprising a random sequence of amino acid residues aspartic acid, glycine, threonine, and glutamic acid (DGTE).

In general, these compositions are synthesized to have a molar ratio of amino acid components of about 1:5:3:1, respectively. Alternatively, the molar ratio of amino acid components is about 1:25:15:5, respectively. Alternatively, the molar ratio of amino acid components is about 1:1:1.5:0.2, respectively.

In another embodiment, any of the copolymers can further comprise an additional amino acid residue, wherein the copolymer has T cell stimulatory activity in a complex with a class II MHC protein, wherein the additional amino acid residue is identified by its position in an autoantigenic peptide for diabetes. For example, the additional amino acid is a lysine residue (K). The K residue is present in sufficient molar ratio to increase T-cell stimulation by the copolymer complexed with a class II MHC protein. Further, the K residue present in sufficient molar ratio to increase aqueous solubility of the copolymer.

The copolymer provided herein is at least about 30 residues in length, at least about 40 residues in length, or the copolymer is at least about 50 residues in length. Further, the copolymer is no greater than about 90 residues in length, no greater than about 80 residues in length, or no greater than about 70 residues in length. The copolymer is synthesized by solution chemistry or by solid phase chemistry.

The copolymer is capable of binding to a class II MHC protein, for example, a human class II MHC protein such as DQ2 or DQ8. Further, the copolymer is capable of binding to a class II MHC protein of a subject animal such as a mouse, for example, IA^{g7} protein. The

composition may be provided in a unit dose, which is effective for treatment of subject for a diabetic condition or celiac disease. The diabetic condition is pre-diabetes; insulin-dependent diabetes mellitus (type I), or type II diabetes. Thus, the diabetic condition is insulin dependent diabetes mellitus. The subject can be a human. Alternatively, the subject is a non-human animal, such as a rodent, such as a rat, mouse or hamster. For example, the subject is a non-diabetic obese (NOD) mouse or a streptozoticin-induced diabetic mouse. The composition in some embodiments further comprises a pharmaceutically acceptable carrier.

The invention in another embodiment provides a method for treating a diabetic condition in a subject, comprising administering to the subject a composition comprising a copolymer having amino acids polymerized in a random sequence, the amino acids comprising at least one residue from a first group of amino acids comprising glutamic acid, aspartic acid, glutamine and asparagine; at least one residue from a second group of amino acids which generally are aliphatic hydrophobic amino acids or hydrophilic hydroxy amino acids and comprises valine, leucine, isoleucine, serine and threonine; and at least one residue from a third group of amino acids which are neutral small residues comprising alanine and glycine, thereby treating the subject for the diabetic condition. In general in the copolymer, the acidic residue is glutamic acid and/or aspartic acid; the neutral residue is alanine and/or glycine; and the aliphatic hydrophobic amino acid is valine, threonine, leucine, and/or isoleucine. In certain embodiments, administering the copolymer is providing the copolymer in a bolus injection, for example, the injection is intravenous (i.v.), subcutaneous (s.c.), intramuscular (i.m.), or intraperitoneal (i.p.). Alternatively, administering the copolymer is providing an intravenous infusion (or drip). In a related embodiment after administering copolymer, the method provides observing a physiological parameter of the diabetic condition. For example, the parameter is decreased free blood glucose, increased blood insulin, increased pancreatic insulin, increased pancreatic mass, or increased number of beta islet cells.

In a related embodiment, the method provides observing a decrease in frequency of diabetic episodes or decrease in severity of diabetic episodes. In a related embodiment of the method, administering the composition is further administering an additional agent. For example, the agent is insulin. The amount of the insulin is less than for the subject prior to administering the copolymer. Alternatively, the additional agent is an immune suppressive agent. The immune suppressive agent can be a drug or a protein. The drug is at least one of a rapamycin; a corticosteroid; an azathioprine; mycophenolate mofetil; a cyclosporine; a

cyclophosphamide; a methotrexate; a 6-mercaptopurine; FK506; 15-deoxyspergualin; an FTY 720; a mitoxantrone; a 2-amino-1,3-propanediol; a 2-amino-2[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride; a 6-(3-dimethyl-aminopropionyl) forskolin; and a demethimmunomycin. The protein is at least one of hul 124; BTI-322; allotrap-HLA-B270; OKT4A; Enlimomab; ABX-CBL; OKT3; ATGAM; basiliximab; daclizumab; thymoglobulin; ISAtx247; Medi-500; Medi-507; Alefacept; efalizumab; infliximab; and an interferon.

Another embodiment of the invention provides a method of manufacture of a medicament for treatment of diabetes or celiac disease, comprising formulating a copolymer having a random sequence of amino acids according to any of the amino and copolymer compositions herein, for administering to a diabetic subject. The embodiment provides a use of a copolymer having a random sequence of amino acids according to any of claims 1-12 for treating a diabetic subject. Another embodiment of the invention provides a kit for treating a diabetic subject comprising a copolymer having a random sequence of amino acids according to any of the amino acid copolymer compositions herein, and a container. The kit can further comprise instructions for use. The kit can provide the copolymer in a unit dose.

Description of Specific Embodiments

Unless the context otherwise requires, as used in this description and in the following claims, the terms below shall have the meanings as set forth:

The term "autoimmune condition" or "autoimmune disease" means a disease state caused by an inappropriate immune response that is directed to a self-encoded entity which is known as an autoantigen. The copolymer compounds provided herein can be used to treat symptoms of an autoimmune disease, a class of disorder which include Hashimoto's thyroiditis; idiopathic myxedema, a severe hypothyroidism; multiple sclerosis, a demyelinating disease marked by patches or hardened tissue in the brain or the spinal cord; myasthenia gravis which is a disease having progressive weakness of muscles caused by autoimmune attack on acetylcholine receptors at neuromuscular junctions; Guillain-Barre syndrome, a polyneuritis; systemic lupus erythematosis; uveitis; autoimmune oophoritis; chronic immune thrombocytopenic purpura; colitis; diabetes; celiac disease which is gluten intolerance; Grave's disease, which is a form of hypothyroidism; psoriasis; pemphigus vulgaris; and rheumatoid arthritis (RA).

The term "diabetes" as used herein means any manifested symptoms of diabetes in any mammal including experimental animal models, and including human forms such as type I

insulin dependent, that is linked genetically to DQ2 and DQ8, type II diabetes, early stage diabetes, and a pre-diabetic condition characterized by mildly decreased insulin or mildly elevated blood glucose levels. A "pre-diabetic condition" describes a mammal suspected of having a diabetic or related condition, for example, not formally diagnosed with diabetes, but demonstrating a symptom in terms of insulin or glucose level, and susceptibility to diabetes or a related condition due to family history, genetic predisposition, or obesity in the case of type II diabetes, or has previously had diabetes or a related condition and is subject to risk of recurrence. While the current diabetes epidemic is primarily type II or adult onset diabetes and is characterized as insulin resistance, damage to beta cells and insulin insufficiency may result.

Another autoimmune disease linked to DQ2 and DQ8 is celiac disease. The term "celiac disease" refers to gluten intolerance resulting in symptoms such as nutritional deficiencies, maladsorption, and distended abdomen. Gluten is found in wheat, barley, oats, and rye, and the disease affects many people worldwide, forcing sufferers to avoid foods that contain these grains. This intolerance is considered to be the most common genetic disease in Europe. Although an estimated one in 4,700 Americans have been diagnosed with this disease, a study suggests that as many as 1 in every 250 Americans may have some form. One effect is damage to the small intestine which is reversible with avoidance of dietary gluten. Because the villi become damaged they are unable to absorb water and nutrients. This causes the celiac patient to be susceptible to a variety of other conditions related to malabsorption. The symptoms of celiac disease can vary with each individual, and range from minimal symptoms to severe gas, bloating, diarrhea, and abdominal pain. If untreated, malnutrition can be life-threatening. Symptoms are not necessarily manifested in the digestive system, and include irritability, depression, muscle cramps, joint pain, fatigue, and menstrual irregularities, to name a few. Celiac disease inheritance has been linked to class II MHC proteins DQ2 and DQ8 (Sollid et al., Gastroenterol. 105: 910, 1993). The same therapeutic application may therefore be useful in this disease as in type I diabetes.

The term "derivative" of an amino acid means a chemically related form of that amino acid having an additional substituent, for example, N-carboxyanhydride group, a γ-benzyl group, an ε,N-trifluoroacetyl group, or a halide group attached to an atom of the amino acid.

The term "analog" means a chemically related form of that amino acid having a different configuration, for example, an isomer, or a D-configuration rather than an L-configuration, or an organic molecule with the approximate size, charge, and shape of the amino acid, or an amino

acid with modification to the atoms that are involved in the peptide bond, so that the copolymer having the analog residue is more protease resistant than an otherwise similar copolymer lacking such analog, whether the analog is interior or is located at a terminus of the copolymer, compared to the copolymer without the analog.

The phrases "amino acid" and "amino acid copolymer" can include one or more components which are amino acid derivatives and/or amino acid analogs as defined herein, the derivative or analog comprising part or the entirety of the residues for any one or more of the 20 naturally occurring amino acids indicated by that composition. For example, in an amino acid copolymer composition having one or more tyrosine residues, a portion of one or more of those residues can be substituted with homotyrosine. Further, an amino acid copolymer having one or more non-peptide or peptidomimetic bonds between two adjacent residues, is included within this definition.

The term "hydrophobic" amino acid means aliphatic amino acids alanine (A, or ala), glycine (G, or gly), isoleucine (I, or ile), leucine (L, or leu), methionine (M, or met), proline (P, or pro), and valine (V, or val), the terms in parentheses being the one letter and three letter standard code abbreviations for each amino acid, and aromatic amino acids tryptophan (W, or trp), phenylalanine (F, or phe), and tyrosine (Y, or tyr). These amino acids confer hydrophobicity as a function of the length of aliphatic and size of aromatic side chains, when found as residues within a copolymer or other polypeptide. The term "hydrophilic hydroxy" amino acid means serine (S) or threonine (T).

The term "charged" amino acid means amino acids aspartic acid (D or asp), glutamic acid (E or glu), arginine (R or arg) and lysine (K or lys), which confer a positive (lys, and arg) or negative (asp, glu) charge at physiological values of pH on an aqueous solution of a copolymer or other amino acid composition containing one or more residues of these amino acids. Histidine (H or his) is hydrophobic at pH 7, and charged at pH 6.

The term "anergy" means unresponsiveness of the immune system of a subject to an antigen.

The term "subject" as used herein indicates a mammal, including a human.

The term "heterologous cell" means a cell for production of an MHC protein which is unrelated to a cell of a subject, e.g., the heterologous cell is not a cell of a mammal. The heterologous cell for example can be from a cold blooded animal, for example, from an

invertebrate; the heterologous cell is an insect cell, or a cell of a microorganism such as a yeast cell.

The term "surfaces of a class II MHC HLA protein" includes the portions of the protein molecule in its three-dimensional configuration which are in contact with its external environment, including those features of the protein that interact with aqueous solvent and are capable of binding to other cell components such as nucleic acids, other proteins, and peptides.

Terms "P1 pocket" and "P4 pocket" include three dimensional polymorphic regions on the peptide binding surface of the Class II MHC protein molecule that accommodate amino acid residue side chains from a peptide that is bound to the class II MHC protein (Fridkis-Hareli, M. et al. 1998. *J. Immunol.* 160:4386-4397; Fridkis-Hareli, M. et. al. 2000. *Human Immunol.* 61:640; Fridkis-Hareli, M. et al. 2001.*Human Immunol.* 62:753-763), including a bound naturally occurring antigen or epitope, and a bound synthetic peptide or copolymer.

The terms "P-1 position" and "P5 position" refer to amino acid residues on the Class II MHC protein molecule peptide complex which directly contact the T-cell receptor (Fridkis-Hareli, M. et. al. 2000. *Human Immunol.* 61:640; Fridkis-Hareli, M. et al. 2001.*Human Immunol.* 62:753-763). The P-1 position refers to the amino acid which precedes the amino acid residue of the peptide that occupies the P1 pocket. The P5 position refers to the amino acid residue that follows the amino acid residue that occupies the P4 pocket in amino acid sequence of a peptide or polypeptide. The P2, P3 and P5 residues are TCR contact residues. Similarly, the P9 position refers to the amino acid residue located four positions beyond the P5 position in amino acid sequence of a peptide or polypeptide.

The term "antigen binding groove" refers to a three dimensional antigen interactive site on the surface of the Class II MHC protein molecule (Stern, L.J. et. al., Nature 368:215 (1994)) that is formed by surfaces of both the α and β subunits of the Class II MHC protein molecule.

The term "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antimicrobials such as antibacterial and antifungal agents, isotonic and absorption delaying agents and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, oral, intraperitoneal, transdermal, or subcutaneous administration, and the active compound can be coated in a material to protect it from inactivation by the action of acids or other adverse natural conditions.

An autoimmune disease results when a host's immune response fails to distinguish foreign antigens from self molecules (autoantigens) thereby eliciting an aberrant immune

response. The immune response towards self molecules in an autoimmune disease results in a deviation from the normal state of self-tolerance, which involves the destruction of T cells and B cells capable of reacting against autoantigens, which has been prevented by events that occur in the development of the immune system early in life. The cell surface proteins that play a central role in regulation of immune responses through their ability to bind and present processed peptides to T cells are the major histocompatibility complex (MHC) molecules (Rothbard, J.B. et al., Annu. Rev. Immunol. 9:527 (1991)).

A "humanized" mouse model of diabetes in which the mice lack endogenous class II genes but transgenetically express human HLA-DQ8 and DR3 proteins is used in the studies herein. GAD65 is injected into these transgenic mice which are thus immunized with GAD65. HLA-DR3 and DQ8 and their bound peptide fragments from GAD65 are purified from mouse spleen and lymph nodes after the appearance of GAD65 antibody. The obtained peptide pool is fractionated and the T-cells generated from the immunized transgenic mice are tested for their response to these peptides. Whether GAD65 peptides are presented by both DR and DQ proteins in these transgenic animals is determined, and the sequences of the peptides associated with each type of protein is compared, to determine whether they are the same or overlapping peptides. Moreover, whether the presence of one class II MHC protein influences the peptide repertoire present associated with the other protein is determined from comparison of single and double transgenic animals.

Purification of these MHC proteins was achieved using the BioCAD instrument which permits a microscale and rapid purification. The peptide pool was fractioned by HPLC and the peptide peaks of interest were identified by IL-2 production or proliferation assays using the T cell hybridomas generated from the spleens and lymph nodes of the immunized transgenic mice, followed by identification of the peptide in peaks. Finally, the peptides identified are synthesized and mice are immunized with them to determine whether they are immunogenic.

Copolymers of amino acids as therapeutic agents for autoimmune diseases

Methods of the invention include use of a class of agents that can bind to class II MHC proteins encoded by particular alleles. Such an agent can bind to a particular class II MHC protein, and thus inhibit and/or prevent the binding of an autoantigen involved in an autoimmune disease, or upon binding can induce anergy, so that there is no response of the immune system to the autoantigen.

A number of therapeutic agents have been developed to treat autoimmune diseases. For example, agents have been developed that can, by inhibiting a cyclooxygenase, prevent formation of low molecular weight inflammatory compounds. Also, agents are available that can function by inhibiting a protein mediator of inflammation, by sequestering the inflammatory protein tumor necrosis factor (TNF) with an anti-TNF specific monoclonal antibody fragment, or with a soluble form of the TNF receptor. Finally, agents are available that target and inhibit the function of a protein on the surface of a T cell (the CD4 receptor or the cell adhesion receptor ICAM-1) thereby preventing a productive interaction with an antigen presenting cell (APC). However, compositions which are natural folded proteins as therapeutic agents can incur problems in production, formulation, storage, and delivery. Further, natural proteins can be contaminated with pathogenic agents such as viruses and prions.

An additional target for inhibition of an autoimmune response is the set of lymphocyte surface proteins represented by the MHC molecules. Specifically, these proteins are encoded by the Class II MHC genes designated as HLA (human leukocyte antigen) -DR, -DQ and -DP. Each of the MHC genes is found in a large number of alternative or allelic forms within a mammalian population. The genomes of subjects affected with certain autoimmune diseases, for example, diabetes, MS and rheumatoid arthritis (RA), are more likely to carry one or more characteristic class II MHC alleles, to which that disease is linked.

The class II MHC protein consists of two approximately equal-sized subunits, α and β, which are transmembrane proteins. A peptide-binding cleft, which is formed by protein features of both α and β subunits, is the site of presentation of the antigen to T cells. There are at least three types of class II MHC molecules: HLA-DR, -DQ, and -DP, and there are numerous alleles of each type. The class II MHC molecules are expressed predominantly on the surfaces of B lymphocytes and antigen presenting cells such as macrophages and dendritic cells (Mengle-Gaw, L., *The Major Histocompatibility Complex* (MHC), in the Encyclopedia of Molecular Biology, Oxford: Blackwell Science Ltd., 1994, pp. 602-606).

An embodiment of the invention includes a novel method for treating autoimmune diseases, by targeting class II MHC molecules with a class of compounds identified as copolymers that include three or more different amino acids.

A copolymer of the invention can be synthesized using Fmoc or t-boc initiating amino acid analogs, or the like, which are immobilized on a resin in an automated peptide synthesis

apparatus for further polymerization (solid state synthesis). The amino acids are polymerized in molar ratios that can be adjusted to provide a copolymer with optimal binding characteristics.

Synthesis procedures can include providing a solution which is a mixture of the chosen amino acids in an activated form, for example, activated as an N-carboxy anhydride, in the appropriate molar ratios of each of the appropriately derivatized amino acid precursors (derivatized to protect certain functional groups, such as the ε amino group of L-lysine, for example the precursor ε,N-trifluoroacetyl-L-lysine). Alternatively, the synthesis procedure can involve online mixing during the synthetic procedure of derivatized precursors of the selected amino acids in the preferred molar ratios. Copolymer synthesis services can be obtained commercially, for example, at Chiron Technologies, Clayton, Australia, the Harvard Medical School Biopolymer Laboratory, Boston, MA, and at Advanced ChemTech, Inc., Louisville, KY.

Examples of such resin supports for peptide synthesis include a Merrifield resin, chloromethylated polystyrene with 1% DVB cross-links; an Fmoc amino acid Wang resin, 4-benzyloxybenzyl alcohol, the resins being pre-loaded with an amino acid (for example, Fmoc-D-trp(boc)-Wang resin). Resins are available in different mesh sizes, for example 100-200 mesh, and high loading or low loading densities of functionalization of the initiating amino acid.

A solution of the different derivatized amino acids to be polymerized into the composition of the invention, preferably protected as conventional in peptide synthesis, is added to sample of beads e.g., Fmoc. Reagents for synthesis, for deblocking, and for cleavage of the complete copolymer molecules for removal from the resin are available from manufacturers of the apparatus (Applied Biosystems Peptide Synthesizer, Foster City, CA, or Advanced ChemTech, Louisville, KY); see e.g., M. Bodansky, *Principles of Peptide Synthesis*, 2nd Ed., Springer-Verlag, 1991, the contents of which are herein incorporated by reference. Additional amino acids or analogs or derivatives of amino acids, can be added to the at least three amino acids selected to comprise the copolymers, to substitute for a small proportion of those amino acids, to provide, for example, a copolymer having increased protease resistance and therefore having enhanced pharmacological properties such as longer in vivo lifetime. Examples of analogs are homotyrosine, or other substituted tyrosine derivatives, and aminobutyric acid, each available as an Fmoc derivative from Advanced ChemTech.

Choice of amino acid residues affecting efficacy of copolymers: design considerations

As discussed herein, while a number of copolymers of different amino acid composition have been found to ameliorate the symptoms of MS and the related mouse model disease EAE,

the molecular mechanism for this effect is not completely clear. One model suggests that the copolymer competes with autoantigenic peptides in formation of a complex with the class II MHC protein (blocking). According to this model, a copolymer having the types of amino acid residues that most closely fit pockets in the protein at key positions or "anchor" residues would be most effective in ameliorating symptoms of the autoimmune disease. For the class II MHC proteins associated with diabetes, the most important positions of a peptide for interaction with protein pockets are P1 and P9. Insights into the structure of the class II MHC protein DQ8 in its interactions with a peptide were obtained from crystallographic studies of a complex with an insulin peptide (Lee et al., Nat. Immunol 2: 501, 2001). The P1 and P9 pockets are considered to be "promiscuous" in that these are large pockets that can accomodate a variety of different amino acid side chains, although the binding is particularly tight when P1 and P9 is glutamic acid (E) or aspartic acid (D).

Binding of a copolymer is not likely to be the only mechanism involved in its immunosuppressive activity. An additional consideration is the nature of interactions of class II MHC protein/peptide or copolymer complexes with T cells, via the T cell receptor (TCR). Differential stimulation may be especially interesting (Vignali, D.A.A. and Strominger, J.L. J. Exp. Med. 179: 1945-1956, 1994). According to this model an interaction with the TCR receptor sufficient to alter the responses of T cells, or to expand existing T cells, may be important to produce or increase production of an immunosuppressive cytokine IL-10. It is likely that the important positions for interaction of class II MHC protein complexes with T cells are P2, P4 and P5. See Wucherpfennig et al., J. Exp. Med. 179: 279, 1994; see also Bettelli et al., J. Immunol. 161: 3299, 1998, and Aharoni et al., J. Neuroimm. 91: 135, 1998, showing stimulation of T cells with mouse class II MHC protein complexes, and Duda et al., J. Cell Immunol. 105: 967, 2000, with human class II MHC protein complexes. Arnon et al. Proc Natl Acad Sci U S A. 100(24):14157-62, 2003, showed that treament with Cop I induces specific Th2 cells in the central nervous system of mice, and that these Th2 cells secrete immunosuppressive cytokines.

Without being limited by any specific mechanism of action, a first group of amino acids was chosen herein that when incorporated into a copolymer will occupy P1 and P9 pockets. The first group of amino acids were chosen on the basis of a number of different criteria, for example, analysis of data shown herein in Table 3, and includes aspartic acid, glutamic acid, asparagine and glutamine. A second group of amino acids are chosen to interact with the TCR

when occupying the P4 position, which can also be promiscuous. See Herman et al., J. Immunol. 163: 6275, 1999. The second group of amino acids are valine, isoleucine, leucine, serine and threonine. Additional amino acids may be used, such as lysing, that affect the charge of the copolymer, and therefore presumably the aqueous solubility, and in addition may when occupying a position in the copolymer, interact with the TCR to alter the response of a T cell.

Therapeutic compositions in the methods of the invention

A pharmaceutically acceptable carrier includes any and all solvents, dispersion media, coatings, antimicrobials such as antibacterial and antifungal agents, isotonic and absorption delaying agents and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, oral, intraperitoneal, transdermal, or subcutaneous administration, and the active compound can be coated in a material to protect it from inactivation by the action of acids or other adverse natural conditions.

The methods of the invention include incorporation of a copolymer into a pharmaceutical composition suitable for administration to a subject. A composition of the present invention can be administered by a variety of methods known in the art as is appreciated by the skilled artisan. The active compound can be prepared with carriers that will protect it against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Many methods for the preparation of such formulations are patented and are generally known to those skilled in the art. See, e.g., *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, Ed., Marcel Dekker, Inc., NY, 1978. Therapeutic compositions for delivery in a pharmaceutically acceptable carrier are sterile, and are preferably stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration.

Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus can be administered, several divided doses can be administered over time, or the dose can be proportionally reduced or increased as indicated by the exigencies of the disease situation.

In general, an embodiment of the invention is to administer a suitable daily dose of a therapeutic copolymer composition that is the lowest effective dose to produce a therapeutic effect, for example, mitigation of symptoms of the autoimmune disease. The therapeutic copolymer compounds of the invention are preferably administered at a dose per subject per day

of at least about 2 mg, at least about 5 mg, at least about 10 mg or at least about 20 mg as appropriate minimal starting dosages. In general, the compound of the effective dose of the composition of the invention can be administered in the range of about 50 to about 400 micrograms of the compound per kilogram of the subject per day.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective dose of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compound of the invention employed in the pharmaceutical composition at a level lower than that required in order to achieve the desired therapeutic effect, and increase the dosage with time until the desired effect is achieved.

In another embodiment, the pharmaceutical composition includes also an additional therapeutic agent. Thus in a method of the invention the pharmaceutical copolymer composition can be administered as part of a combination therapy, i.e. in combination with an additional agent or agents. Examples of materials that can be used as combination therapeutics with the copolymers for treatment of autoimmune disease and diabetic conditions as additional therapeutic agents include: an antibody or an antibody fragment that can bind specifically to an inflammatory molecule or an unwanted cytokine such as interleukin-6, interleukin-8, granulocyte macrophage colony stimulating factor, and tumor necrosis factor-α; an enzyme inhibitor which can be a protein, such as α_1-antitrypsin, or aprotinin; an enzyme inhibitor which can be a cyclooxygenase inhibitor; an engineered binding protein, for example, an engineered protein that is a protease inhibitor such an engineered inhibitor of a kallikrein; an antibacterial agent, which can be an antibiotic such as amoxicillin, rifampicin, erythromycin; an antiviral agent, which can be a low molecular weight chemical, such as acyclovir; a steroid, for example a corticosteroid, or a sex steroid such as progesterone; a non-steroidal anti-inflammatory agent such as aspirin, ibuprofen, or acetaminophen; an anti-cancer agent such as methotrexate or adriamycin; a cytokine blocking agent; an adhesion molecule blocking agent; a cytokine; an immune suppressing drug; and an immune suppressing agent.

A therapeutic agent to be used with the composition of the invention can be an engineered binding protein, known to one of skill in the art of remodeling a protein that is covalently attached to a virion coat protein by virtue of genetic fusion (Ladner, R. et al., U.S. Patent 5,233,409; Ladner, R. et al., U.S. Patent 5,403,484), and can be made according to methods known in the art. A protein that binds any of a variety of other targets can be

engineered and used in the present invention as a therapeutic agent in combination with a copolymer of the invention.

An improvement in the symptoms as a result of such administration is noted by a decrease in frequency of recurrences of episodes of diabetes, by decrease in severity of symptoms, and by elimination of recurrent episodes for a period of time after the start of administration. A therapeutically effective dosage preferably reduces symptoms and frequency of recurrences by at least about 20%, for example, by at least about 40%, by at least about 60%, and by at least about 80%, or by about 100% elimination of one or more symptoms, or elimination of recurrences of the autoimmune disease, relative to untreated subjects. The period of time can be at least about one month, at least about six months, or at least about one year.

Random synthetic copolymers can be used to treat other autoimmune diseases that are associated with HLA-DQ gene products by competing with candidate autoantigens for binding to these protein receptor molecules, or by inducing T cell anergy or even T cell apoptosis, or by suppression of T cells, such that subsequent T cell response to an autoantigen is inhibited in vivo. Further, synthetic copolymers having one or more additional components, such as amino acid analogs or derivatives added in varying quantities into the polymerization reaction, can be effective inhibitors of a variety of autoimmune T cell responses. See PCT/US02/31399 by Strominger et al., and Fridkis-Hareli et al. J. Clin. Invest. 109: 1635-1643, 2002, the entire contents of both of which are hereby incorporated herein by reference.

A major goal in the treatment of autoimmune diseases has been development of antigen-specific immunomodulating therapies that interfere with the trimolecular interaction of the autoreactive T cell receptor (TCR) with the autoantigenic peptides presented by self MHC receptors at the surface of antigen-presenting cells. These immunotherapies of T cell-mediated autoimmune diseases have been successful in animal models with known target antigens (see, for example, Weiner, H.L. 1997. *Immunol. Today* 18:335-343; Nicholson, L.B. et al. 1997. *Proc. Natl. Acad. Sci. USA* 94:9279-9284). The use of altered peptide ligands (APL) has been used both to treat EAE (Nicholson, L.B. et al. 1997. *Proc. Natl. Acad. Sci. USA* 94:9279-9284; Brocke, S. et al. 1996. *Nature* 379:343-346) and recently to treat MS (Bielekova, B. et al. 2000. *Nat. Med.* 10:1167-1175; Kappos, L. et al. 2000. *Nat. Med.* 10:1176-1182), with contradictory findings.

The invention having been fully described, it is illustrated by the following examples and claims, which are exemplary and are not to be construed as further limiting. The contents of all references cited herein are incorporated by reference.

EXAMPLES

Materials and Methods

Copolymers, peptides and antibodies. Peptides were synthesized using solid phase techniques (Barany, G., and R. Merrifield. 1979. *Academic Press*, New York, NY) on an Applied Biosystems Peptide Synthesizer and purified by reversed-phase HPLC(RP-HPLC).

Protein expression and purification. Soluble HLA-Dr-Q molecules were expressed in *Drosophila* S2 cells and purified as described (Kalandadze, A. et al. 1996. *J. Biol. Chem.* 271:20156-20162). Cells were grown at 26°C in roller bottles in ExCell 401 medium (JRH Biosciences, Lenexa, KS) supplemented with 0-5% fetal bovine serum (Sigma Chemicals, St. Louis, MO). Cells were harvested 4-5 days after induction by 1 mM $CuSO_4$. Supernatant from harvested cells was sequentially passed through Protein A, Protein G and Protein A-LB3.1 columns, followed by elution of the bound HLA-DR with 50 mM 3-[cyclohexylamino]-1-propanesulfonic acid (CAPS), pH 11.5, and neutralized with 200 mM phosphate (pH 6.0). Proteins were concentrated on a Centriprep 10 membrane (Amicon, Beverly, MA).

HPLC separation and microsequencing. Different copolymers are separated and pool sequenced as previously described (Fridkis-Hareli, M. et al. 1999. *J. Immunol.* 162:4697-4704). Briefly, the fractionation is by microbore HPLC using a Zorbax C_{18} 1.0 mm reverse-phase column on a Hewlett-Packard 1090 HPLC with 1040 diode array detector. Copolymers are eluted at a flow rate of 54 µl/min with a gradient of 0.055% trifluoroacetic acid (TFA) in acetonitrile (0% at 0 to 10 min, 33% at 73 min and 60% at 105 min). Strategies for peak selection, reverse phase separation and Edman microsequencing have been previously described (Chicz, R.M. et al. 1993. J. Exp Med.178: 27-47; Godkin, A. et al. Int Immunol. 1997 9:905-11). Pooled fractions are submitted to automated Edman degradation on a Hewlett-Packard G1005A (Palo Alto, CA) protein sequencer using the manufacturer's Routine 3.5.

Assays for peptide binding to class II MHC proteins.

(A). Solutions. The solutions used in this assay are the following: binding buffer is 20 mM 2-[N-morpholino]ethanesulfonic acid (MES), 140 mM NaCl, 0.05% NaN_3, pH 5.0, unless otherwise specified; PBS is 150 mM sodium chloride, 7.5 mM sodium phosphate, dibasic, 2.5 mM sodium phosphate, monobasic, pH 7.2; TBS is 137 mM sodium chloride, 25 mM Tris pH 8.0, 2.7 mM potassium chloride; TTBS is TBS plus 0.05% Tween-20.

(B). Microtiter assay plate preparation. Immunoassay plates (96-well microtiter, PRO-BIND™, Falcon, Lincoln Park, NJ) were coated with 1 μg/well affinity-purified LB3.1 monoclonal antibodies in PBS (100 μl total) for 18 hrs at 4°C. The wells were then blocked with TBS/3% BSA for 1 hr at 37°C and washed three times with TTBS. Before sample addition, 50 μl of TBS/1% BSA was added to each well.

(C). Inhibition reactions. Biotinylated peptide, final concentration 0.13 μM in 50 μl of the binding buffer, is co-incubated with unlabeled inhibitors (random copolymers or control peptides), and HLA-DQ molecules for 40 hr at 37°C.

(D). Detection of class II MHC protein/peptide complexes. Bound peptide-biotin was detected using streptavidin-conjugated alkaline phosphatase, as follows. Plates were washed three times with TTBS and incubated with 100 μl of streptavidin-conjugated alkaline phosphatase (1:3000, BioRad, Richmond, CA) for 1 hr at 37°C, followed by addition of p-nitrophenyl phosphate in triethanolamine buffer (BioRad). Absorbance at 410 nm was monitored by a microplate reader (model MR4000; Dynatech, Chantilly, VA).

Mouse strains. Non-obese diabetic mice are obtained from Jackson Laboratories, Bar Harbor, ME.

Example 1. Peptides bound to human class II MHC HLA-DQ8, small-scale isolation

Techniques were used for sequencing of peptides eluted from HLA-DQ8 purified on a small scale. Purification from HLA-DQ8 protein isolated from 1g of cells. Priess cells (HLA-DR4, HLA-DQ8 homozygous) are the standard in this investigation. POROS immunoaffinity columns (BioCAD instrument) were used to isolate HLA-DQ8 (as well as HLA-DR4) from 20 grams of cells. Peptides were eluted from 1/20 of this material (equivalent of to 1g of cells).

A total of 79 peptide sequences were identified in the mixture by LC-MS-MS (Table 1). Many of these represented nested peptide sets. One peptide in particular derived from a Class I MHC protein was present in 10 different sequences differing only at the N- or C- terminus of the peptide. The core in this set was readily identified. This represents the largest number of

peptides isolated from HLA-DQ8 protein and identified, and this critical number provides a consensus for analysis of such sites on the protein as the P1 and P9 positions (see Table 3). In the previous two studies of HLA-DQ8 (1, 2), sequences of eight peptides were reported in one and the other described only peptide pool sequencing. This analysis was then used with one gram of Priess cells for isolation (1 gram is the amount of spleen that can be obtained from 15-20 mice.). The HLA-DQ8 protein complex having endogenous peptide was readily isolated on this scale, and peptides were readily identified (Table 2). This procedure yielded the sequences of 112 peptides, many more than were previously observed (Chicz RM, et al.. Int. Immunol. 1994 6:1639-49; Godkin, A. et al., Int. Immunol. 9:905, 1997). On the basis of such a large number of sequences, it is possible to develop a consensus for binding by the class II MHC DQ2 and DQ8 proteins.

Example 2. Analysis of peptide sequences

The eluted peptides were found to be generally acidic, with surprising overrepresentation of both aspartic acid (D) and glutamic acid (E). Alignment of the peptides with E or D near the carboxy terminus of the core, i.e., at P9, is shown in Table 3. A preference for an acidic amino acid at P1 was also evident in the alignment but it maybe less striking than that observed with mouse protein I-Ag7 (Suri A, et al. J Immunol. 2002 168(3):1235-43). The distinction between preferences from one species to another may be especially significant for immune recognition.

Example 3. Analysis of peptide bound to mouse class II MHC

Experiments are conducted to obtain a similar analysis, using I-Ag7 isolated from mouse spleens, in contrast to a published procedure using 15 mouse spleens, and that resulted in the isolation of a small amount of Iag7 of high purity (Suri et al., J Immunol 168 (3): 1235-43, 2002). Peptides are isolated, sequenced, and analyzed as shown above for human class II MHC proteins, to obtain a consensus for the mouse. The microtechnique for isolation of I-Ag7 and purification of peptides is performed as for HLA-DQ8. The above example is conducted with the HLA-DQ8 transgenic mouse and the HLA-DR3/HLA-DQ8 double transgenic mouse. The BDC2.5 TCR, CDld-/- mouse in which the disease develops much more rapidly (Shi, F.D. et al. Proc. Natl. Acad. Sci. USA 98: 6777-6782, 2001) is also employed.

Example 4. Copolymers for the treatment of diabetes: Copaxone control.

To develope a copolymer that could prevent progression of diabetes in the NOD mouse, the consensus amino acids observed for P1 and P9 positions herein in Table 3 were used as the

basis of choice of amino acid residues, i.e., which amino acids to use to obtain a copolymer having a random sequence.

NOD mice are used as an experimental system (Shi, F.D., et al. Proc. Natl. Acad. Sci. USA 98: 6777-6782, 2001). These mice begin to develop diabetes at about 13-15 weeks and data are obtained after about 30 weeks to obtain data, such as a frequency of symptoms for comparison of treated and untreated groups of mice. NOD mice were treated herein with Copaxone, and no difference was found between the untreated animals and animals treated with Copaxone. In the experiment, 10μg of Copaxone was injected into mice three times a week (equivalent to the human dose on a weight ratio basis. A higher dose, 33μg Copaxone/mouse three times a week, is also employed. These experiments test the hypothesis that Copaxone would be ineffective.

New copolymers based on the binding motif in Table 3 are designed, synthesized, and tested. The composition of these copolymers is given in the Summary herein.

Example 5. Analysis of GAD65 peptides

The data to be obtained relate to understanding of presentation of GAD peptides to T cells by DR3 and DQ8 proteins complexed with the peptides, and will provide further information on the DQ8 binding motif from the pool of peptide sequence. Induction of tolerance to autoimmune diseases by administration of copolymers and/or auto antigenic peptides is a topic of great practical as well as theoretical interest. Identification of peptides derived from GAD65 and presented by DR and DQ proteins will allow design of amino acid-based human therapeutics.

Data herein describe isolated naturally processed peptides that are bound to HLA-DQ8. Further work will analyze peptides to be obtained from HLA-DQ8 and HLA-DR3 protein in spleens and lymph nodes of Aß°/DQ3, DQ8 transgenic mice that develop insulitis and are immunized with GAD65 or display GAD65 autoreactivity spontaneously.

The immune system is able to distinguish foreign molecules from the endogenous or "self" cell components such as proteins. Once a foreign molecule is recognized, the immune system enlists participation of a variety of cells (e.g. B and T cells) and molecules to mount an appropriate response to eliminate them. Autoimmunity occurs when an immune response is mounted against self-components. The protein to be recognized by T cells is cleaved, for example, proteolytically, into small fragments (peptides), which are then associated with major histocompatibility complex (MHC) molecules and transported to the cell surface. Examples of

MHC molecules are HLA-DR4, DQ8, DR3, and DQ2, certain alleles of which have been shown to be associated with the greater risk for insulin-dependent diabetes mellitus (IDDM). IDDM is thought to be a T cell-mediated autoimmune disease in which T cells destroy the insulin-secreting ß cells of the pancreatic islets of Langerhans. The peptides derived from glutamic acid decarboxylase (GAD65) an enzyme mainly restricted to brain and ß cells found in pancreatic islets, have been implicated in the pathogenesis of the disease.

A humanized mouse model of diabetes in which the mice lack endogenous class II genes and instead transgenically express human HLA-DQ8 and DR3 proteins, is used herein. GAD65 is injected into these transgenic mice which are then immunized with GAD65.

Microscale purification of DR3 and DQ8 proteins will be used to obtain these proteins, using the purification procedure that was optimized above using human B cell lines WT20 and Priess cells. This procedure is used applying the materials from transgenic mice. The peptide pool was fractionated and the sequence of the peptides was analyzed. T cell hybridomas are generated from the spleens and lymph nodes of immunized transgenic mice and tested for their response to these peptides. This material is likely to contain several different peptides. The GAD65 peptides are identified from amino acid sequence data, using sequences to be obtained from several peptides in each pool. Finally, the peptides identified are synthesized and mice are immunized with them to determine whether these are immunogenic.

Presentation of GAD65 peptides to T cells by DR3 and DQ8 proteins provides further information on the DQ8 binding motif, by comparison of amino acid sequences of peptides from the pool. Data obtained is used to design the copolymers herein. The definitive identification of peptides derived from GAD65 and presented by DR and DQ proteins could therefore, have important practical consequences for human therapeutics.

Table 1. Peptide sequencess obtained from Priess cell preparation (20 grams, 1/20 run on LCMSMS)

Proteoglycan 1

1. LPSDSQDLGQHGLEE*DFM** (8 nested) (SEQ ID NO: 10)
2. IQDLNRIFPLSEDYS (SEQ ID NO: 11)

Class I histocompatibility antigen

3. TAADTAAQITQRK*W* (3 nested) (SEQ ID NO: 12)

4. GPEYWDRETQISKTN*TQ* (3 nested) (SEQ ID NO: 13)
5. *PAGDRTFQK*WAAVVVPSGEE*QR* (10 nested) (SEQ ID NO: 14)
6. *DTQFVR*FDSDAASPR*GEP* (3 nested) (SEQ ID NO: 15)

MHC class I antigen

7. LNEDLRSWTAADTAA (SEQ ID NO: 16)

MHC class antigen (A30.2 alpha chain)

8. VRFDSDAASQRME*PRAP* (4 nested) (SEQ ID NO: 17)

Ig kappa chain variable region

9. IPDRFSGSGSGTDF*TLT* (4 nested) (SEQ ID NO: 18)
10. *GS*GTDFTLTISRLEPED*F* (4 nested) (SEQ ID NO: 19)

Glyceraldehyde-3-phosphate dehydrogenase

11. *ST*FDAGAGIALNDH (3 nested) (SEQ ID NO: 20)

Trans-golgi network integral membrane protein TGN51 precursor

12. GPIDGPSKSGAEE (SEQ ID NO: 4)
13. TGPEEGSPPKEEKE (SEQ ID NO: 21)
14. TPKDGSNKSGAEEQGPI (SEQ ID NO: 22)

HLA-DR alpha heavy chain

15. *K*FHYLPFLPSTE*DVYD* (3 nested) (SEQ ID NO: 23)

Unknown

16. DPGGSVPSGE*AS* (3 nested) (SEQ ID NO: 24)

Unnamed protein product

17. *LVKGFY*PSDIAVEWE*SN* (4 nested) (SEQ ID NO: 25)

CD44 antigen

18. DGPITITIVNRDG*TR* (2 nested) (SEQ ID NO: 26)

CD74 antigen invariant polypeptide

19. PSSGLGVTKQDLGPV*M** (3 nested) (SEQ ID NO: 27)

P60 antigen

20. DEDGDLVAFSSDEE (SEQ ID NO: 28)

21. RPGTAESASGPSEDPSVN (SEQ ID NO: 29)

Beta-2-microglobulin precursor

22. LYYTEFTPTEKDEYA*C* (2 nested) (SEQ ID NO: 30)

Solute carrier family

23. GPAGDATVASEKE*S* (2 nested) (SEQ ID NO: 31)

Similar to WW domain binding protein 2

24. GPDVPSTAAE*AKA* (2 nested) (SEQ ID NO: 32)

Heat shock cognate 71 kDa Protein

25. INWLDKNQTAEKEEFEH (2) (SEQ ID NO: 33)

26. TGIPPAPRGVPQ (SEQ ID NO: 34)

MHC class I antigen (peromyscus maniculatus)

27. FQKWAAVVVPTGEE (2 nested) [only differs by S -> T difference from above class I] (SEQ ID NO: 35)

MHC DQ-alpha 1 protein (rhesus macaque)

28. FPGDEEFYVDLERKET (2) (SEQ ID NO: 36)

Myobrevin (vesicle associated membrane protein 5)

29. APRTQDAGIASPGPGN (SEQ ID NO: 37)

Fc fragment of IgE, low affinity ii, receptor (CD23A)

30. AQKSQSTQISQELEE (SEQ ID NO: 38)

Table 2. Peptide sequences obtained from Priess cell preparation (1 gram prep)

Proteoglycan 1

1. *SLDRNLPS*DSQDLGQHGLEED*FM***L* (15 nested) (SEQ ID NO: 39)

Class I histocompatibility antigen

2. *RPAGDGTFQ*KWAAVVVPSGEE*QR* (10 nested) (SEQ ID NO: 40)
3. GPEYWDRETQISKTNT (SEQ ID NO: 41)
4. LNEDLSSWTAADTAA (SEQ ID NO: 42)

P60

5. *YRD*EDGDLVAFSSDE*ELT* (6 nested) (SEQ ID NO: 43)

Calnexin

6. IDIEDDLDDVIEEVEDSKP (2) (SEQ ID NO: 44)
7. *KPD*DWDEDAPAKIPDEE (5 nested) (SEQ ID NO: 45)
8. KPEDWDEDM*DGEWEAPG (SEQ ID NO: 46)

Alzheimer's disease amyloid A4 protein homolog precursor

9. *AD*GSEDKVVEVAEEE*EVA* (7 nested) (SEQ ID NO: 47)

Chain A, rabbit serum transferrin

10. APEEGYLSVAVVK (SEQ ID NO: 48)
11. DLGDVAFVK (2) (SEQ ID NO: 49)
12. SGDFQLFSSPHGK (SEQ ID NO: 50)
13. SQTVLQNTGGR (SEQ ID NO: 51)
14. EGYYGYTGAFR (SEQ ID NO: 52)

Fc fragment of IgE, low affinity IL receptor for (CD23A)

15. *HHGDQM*AQ*KSQSTQISQELEE*LRAEQQ* (5 nested) (SEQ ID NO: 53)

Heat shock protein gp 96 precursor

16. *K*EESDDEAAVEEEEEE*KKP* (4 nested) (SEQ ID NO: 54)

17. IDPDAKVEEEPEEEPE (SEQ ID NO: 55)

Class II antigen alpha

18. *F*DGDEEFYVDLERKE*TV* (8 nested) (SEQ ID NO: 56)

19. EDIVADHVASYGNLYQSYGPSGQYSHEFD (3) (SEQ ID NO: 57)

Ig kappa chain variable region

20. *S*GTDFTLTISRLEPED*F* (4 nested) (SEQ ID NO: 58)

Calreticulin precursor

21. *EP*EEEDVPGQAKDEL (2 nested) (SEQ ID NO: 50)

22. IPDPDAKKPEDWDEEM*DGEWEPP (5) (SEQ ID NO: 60)

N-acetyglucosaminyltransferase III

23. *RPDDVF*IIDDADEIPARDG*V* (4 nested) (SEQ ID NO: 61)

DKFZ566HO73 protein

24. GPGDEDQEEETQGQEEGDE*GEPRDHPA* (4 nested) (SEQ ID NO: 62)

CD20 receptor

25. EKKEQTIEIKEEVVG (SEQ ID NO: 63)

26. *PKNEE*DIEIIPIQEEEEE (3 nested) (SEQ ID NO: 64)

Heat shock cognate 71 kDa protein

27. INWLDKNQTAEKEEFE*H* (3 nested) (SEQ ID NO: 65)

Unknown (protein for MGC:12802)

28. ENPEDEPLGPEDEDSFS (SEQ ID NO: 66)

29. EPLGPEDEDSFSNAESYE (SEQ ID NO: 67)

CA125 ovarian carcinoma antigen

30. *IH*IAEEEAVM*EEEEDEE (SEQ ID NO: 68)

Ubiquitin (*Drosophila*)

31. EPSDTXENVKAKIQDKE*G* (SEQ ID NO: 69)

DP alpha chain precursor, similar to

32. *EF*DEDEM*FYVDLDKKET (SEQ ID NO: 70)

Heat shock cognate 70.ii (*Xenopus*)

33. VVSWLDKNQTAEKEEFEH (SEQ ID NO: 71)

Protein disulfide isomerase related protein (calcium-binding protein, intestinal-related)

34. SNRENAIEDEEEEEEE (SEQ ID NO: 72)

Similar to Y39E4B.7.p

35. DPGIFPRAEEDED (SEQ ID NO: 73)

Class I histocompatibility Aw68.1

36. LYYTEFTPTEKDEYA (SEQ ID NO: 74)

MHC surface glycoprotein

37. RKFHYLPFLPSTEDVYD (SEQ ID NO: 75)

Similar to Ig heavy chain 4 (serum IgG1) (mouse)

38. PEVQFSWPVDDVEVHTA (SEQ ID NO: 76)

Table 3. Alignment of sequences of selected peptides obtained from complexes with HLA-DQ8 in the homozygous Priess cell line

Core Peptide	Source Protein	SEQ ID NO	# of nested peptides
LPSDS**QD**LGQHGL**EE**	proteoglycan 1	1	23
W**AA**VVVPSG**EE**	class I MHC	2	20
F**DA**GAGIALN**D**H	glyceraldehyde-3-phosphate dehydrogenase	3	3
GP**ID**GPSKSGA**EE**	trans-golgi network protein (TGN 51)	4	1
DEDG**DL**VAFSSD**EE**	p60	5	7
INWL**D**KNQTAEK**E**EFEH	71 kD heat shock protein	6	5
LYY**TE**FTPTEK**DE**YA	beta-2-microglobulin	7	2
DW**DE**DAPAKI**PDEE**	calnexin	8	5
GS**ED**KVVEVA**EE**E	amyloid A4 protein homolog	9	7

Putative P1 and P9 residues are in bold type. Possible multiple candidates are shown for most of these peptides.

What is claimed is:

1. A copolymer composition comprising a random sequence of at least three different amino acid residues, wherein at least one amino acid is selected from each of: a first group of amino acids consisting of glutamic acid (E), aspartic acid (D), glutamine (Q) and asparagine (N); a second group of amino acids consisting of amino acids valine (V), leucine (L), threonine (T), serine (S) and isoleucine (I); and a third group of amino acids consisting of alanine (A) and glycine (G).

2. The composition according to claim 1, wherein the copolymer is capable of binding to a class II MHC protein.

3. The composition according to claim 2, wherein the copolymer is a terpolymer selected from the group consisting of amino acid residues: aspartic acid:alanine:leucine (DAL); aspartic acid:alanine:valine (DAV); aspartic acid:alanine:threonine (DAT); aspartic acid:alanine:serine (DAS); asparagine:alanine:leucine (NAL); asparagine:alanine:valine (NAV); asparagine:alanine:threonine (NAT); asparagine:alanine:serine (NAS); glutamic acid:alanine:leucine (EAL); glutamic acid:alanine:valine (EAV); glutamic acid:alanine: threonine (EAT); glutamic acid:alanine: serine (EAS); glutamine:alanine:leucine (QAL); glutamine:alanine:valine (QAV); glutamine:alanine: threonine (QAT); glutamine:alanine: serine (QAS); aspartic acid:alanine:isoleucine (DAI); glutamic acid:alanine:isoleucine (EAI); aspartic acid:glyine:isoleucine (DGI); aspartic acid:glyine: threonine (DGT); aspartic acid:glyine: serine (DGS); asparagine:alanine:isoleucine (NAI); glutamine:alanine:isoleucine (QAI); asparagine:glyine:isoleucine (NGI); asparagine:glyine: threonine (NGT); asparagine:glyine: serine (NGS); glutamic acid:glyine:isoleucine (EGI); aspartic acid:glyine:valine (DGV); glutamic acid:glyine:valine (EGV); glutamic acid: glyine:leucine (EGL); glutamic acid: glyine:threonine (EGT); glutamic acid: glyine:serine (EGS); glutamic acid: glyine:valine (EGV); glutamine:glyine:isoleucine (QGI); asparagine:glyine:valine (NGV); glutamine:glyine:valine (QGV); glutamine: glyine:leucine (QGL); glutamine: glyine:threonine (QGT); glutamine:glyine:serine (QGS); and glutamine:glyine:valine (QGV).

4. The composition according to claim 3, wherein the copolymer is a tetrapolymer selected from the group consisting of amino acid residues aspartic acid:alanine:leucine:glutamic acid (DALE); asparagine:alanine:leucine:glutamic acid (NALE); aspartic acid:alanine:leucine:glutamine (DALQ); aspartic acid:alanine:valine: glutamic acid (DAVE); asparagine:alanine:valine: glutamic acid (NAVE); aspartic acid:alanine:valine: glutamine (DAVQ); aspartic acid:alanine:isoleucine:glutamic acid (DAIE); asparagine:alanine:isoleucine:glutamic acid (NAIE); aspartic acid:alanine:isoleucine:glutamine (DAIQ); aspartic acid:alanine:threonine:glutamic acid (DATE); asparagine:alanine:threonine:glutamic acid (NATE); aspartic acid:alanine:threonine:glutamine (DAQE); aspartic acid:alanine:serine:glutamic acid (DASE); asparagine:alanine:serine:glutamic acid (NASE); aspartic acid:alanine:serine:glutamine (DASQ); aspartic acid:glyine:isoleucine:glutamic acid (DGIE); asparagine:glyine:isoleucine:glutamic acid (NGIE); aspartic acid:glyine:isoleucine:glutamine (DGIQ); glutamic acid:glyine:leucine:glutamic acid (DGLE); glutamine:glyine:leucine:glutamic acid (QGLE); aspartic acid:glyine:leucine:glutamine (DGLQ); aspartic acid:glyine:threonine: glutamic acid (DGTE); asparagine:glyine:threonine:glutamic acid (NGTE); aspartic acid:glyine:threonine: glutamine (DGTQ); aspartic acid:glyine:serine: glutamic acid (DGSE); asparagine:glyine:serine: glutamic acid (NGSE); aspartic acid:glyine:serine: glutamine (DGSQ); aspartic acid:glyine:valine:glutamic acid (DGVE). asparagine:glyine:valine:glutamic acid (NGVE); and aspartic acid:glyine:valine:glutamine (DGVQ).

5. A copolymer composition comprising a random sequence of amino acid residues aspartic acid, alanine, leucine, and glutamic acid (DALE).

6. A copolymer composition comprising a random sequence of amino acid residues aspartic acid, alanine, valine, and glutamic acid (DAVE).

7. A copolymer composition comprising a random sequence of amino acid residues aspartic acid, alanine, threonine, and glutamic acid (DATE).

8. A copolymer composition comprising a random sequence of amino acid residues aspartic acid, alanine, isoleuine, and glutamic acid (DAIE).

9. A copolymer composition comprising a random sequence of amino acid residues aspartic acid, glycine, leucine, and glutamic acid (DGLE).

10. A copolymer composition comprising a random sequence of amino acid residues aspartic acid, glycine, valine, and glutamic acid (DGVE).

11. A copolymer composition comprising a random sequence of amino acid residues aspartic acid, glycine, isoleucine, and glutamic acid (DGIE).

12. A copolymer composition comprising a random sequence of amino acid residues aspartic acid, glycine, threonine, and glutamic acid (DGTE).

13. The composition according to any of claims 5-12 wherein the molar ratio of amino acid components is about 1:5:3:1.

14. The composition according to any of claims 5-12, wherein the molar ratio of amino acid components is about 1:25:15:5.

15. The composition according to any of claims 5-12, wherein the molar ratio of amino acid components is about 1:1:1.5:0.2.

16. The composition according to any of claims 1-12, the copolymer further comprising an additional amino acid residue, wherein the copolymer has T cell stimulatory activity in a complex with a class II MHC protein, wherein the amino acid residue is identified by its position in an autoantigenic peptide for diabetes or celiac disease.

17. The composition according to claim 16, wherein the additional amino acid residue is a lysine residue (K).

18. The composition of claim 17, wherein the K residue further increases aqueous solubility of the copolymer.

19. The composition according to any of claims 1-12, wherein the copolymer is at least about 30 residues in length.

20. The composition according to any of claims 1-12, wherein the copolymer is at least about 50 residues in length.

21. The composition according to any of claims 1-12, wherein the copolymer is no greater than about 70 residues in length.

22. The composition according to any of claims 1-12, wherein the copolymer is no greater than about 90 residues in length.

23. The composition according to any of claims 1-12, wherein the copolymer is synthesized by solution chemistry or by solid phase chemistry.

24. The composition according to any of claims 1-12, wherein the molar ratio of the amino acid components is about 2:5:3 for relative amounts of the acidic amino acids: aliphatic aliphatic amino acid: and A or G, respectively.

25. The composition according to any of claims 1-12, wherein the molar ratio of the amino acid components is about 2:25:15 for relative amounts of the acidic amino acids: aliphatic aliphatic amino acid: and A or G, respectively.

26. The composition according to any of claims 1-12, wherein the molar ratio of the amino acid components is about 2:1:0.6 for relative amounts of the acidic amino acids: aliphatic aliphatic amino acid: and A or G, respectively.

27. The composition according to any of claims 1-12 wherein the copolymer is capable of binding to class II MHC protein DQ2 or DQ8.

28. The composition according to any of claims 1-12 wherein the copolymer is capable of binding to class II MHC protein IA^{g7}.

29. The composition according to any of claims 1-12 present in a unit dose effective for treatment of subject for a diabetic condition.

30. The composition according to claim 29, wherein the diabetic condition is insulin dependent diabetes mellitus or celiac disease.

31. The composition according to claim 29, wherein the subject is a human.

32. The composition according to claim 29, wherein the subject is a rodent.

33. The composition according to claim 32, wherein the subject is a non-diabetic obese (NOD) mouse or a streptozoticin-induced diabetic mouse.

34. The composition according to any of claims 1-12, further comprising a pharmaceutically acceptable carrier.

35. A method for treating a diabetic condition or celiac disease in a subject, comprising administering to the subject a composition comprising a copolymer having amino acids polymerized in a random sequence, the amino acids comprising at least one acidic residue, at least one neutral small residue, and at least one aliphatic hydrophobic amino acid, thereby treating the subject for the diabetic condition or the celiac disease.

36. The method according to claim 35, wherein the acidic residue is glutamic acid and/or aspartic acid; the neutral residue is alanine and/or glycine; and the aliphatic hydrophobic amino acid is valine, threonine, leucine, and/or isoleucine.

37. The method according to claim 35, wherein administering the copolymer is providing the copolymer in a bolus injection.

38. The method according to claim 37, wherein the injection is selected from the group of: intravenous (i.v.), subcutaneous (s.c.), intramuscular (i.m.), and intraperitoneal (i.p.).

39. The method according to claim 37, wherein administering the copolymer is providing an intravenous infusion.

40. The method according to claim 35, further comprising after administering copolymer, observing a physiological parameter of the diabetic condition or celiac disease.

41. The method according to claim 40, wherein the parameter is decreased free blood glucose, increased blood insulin, increased pancreatic insulin, increased pancreatic mass, and increased number of beta islet cells.

42. The method according to claim 35, further comprising after administering copolymer, observing a decrease in frequency of diabetic episodes or decrease in severity of diabetic episodes.

43. The method according to claim 35, wherein the diabetic condition is selected from the group of: pre-diabetes; insulin-dependent diabetes mellitus (type I), and type II diabetes.

44. The method according to claim 35, wherein the diabetic condition is insulin-dependent diabetes mellitus (type I).

45. The method according to claim 35, wherein administering the composition is further administering an additional agent.

46. The method according to claim 45, wherein the agent is insulin.

47. The method according to claim 46, wherein the amount of the insulin is less than for the subject prior to administering the copolymer.

48. The method according to claim 45, wherein the agent is an immune suppressive agent.

49. The method according to claim 48, wherein the agent is a drug or a protein.

50. The method according to claim 49, wherein the drug is at least one of a rapamycin; a corticosteroid; an azathioprine; mycophenolate mofetil; a cyclosporine; a cyclophosphamide; a methotrexate; a 6-mercaptopurine; FK506; 15-deoxyspergualin; an FTY 720; a mitoxantrone; a 2-amino-1,3-propanediol; a 2-amino-2[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride; a 6-(3-dimethyl-aminopropionyl) forskolin; and a demethimmunomycin.

51. The method according to claim 49, wherein the protein is hul 124; BTI-322; allotrap-HLA-B270; OKT4A; Enlimomab; ABX-CBL; OKT3; ATGAM; basiliximab; daclizumab; thymoglobulin; ISAtx247; Medi-500; Medi-507; Alefacept; efalizumab; infliximab; and an interferon.

52. A method of manufacture of a medicament for treatment of diabetes or celiac disease, comprising formulating a copolymer having a random sequence of amino acids according to any of claims 1-12 for administering to a diabetic subject.

53. A use of a copolymer having a random sequence of amino acids according to any of claims 1-12 for treating a diabetic subject.

54. A kit for treating a diabetic subject comprising a copolymer having a random sequence of amino acids according to any of claims 1-12 and a container.

55. The kit of claim 54, further comprising instructions for use.

56. The kit of claim 54, in a unit dose.

Abstract

Naturally processed peptides bound to HLA-DQ8 were obtained and sequenced individually. A consensus is used to design copolymers for treatment of an autoimmune condition such as diabetes.

5